

ABSTRACT

U.S.-Canadian Academy of Pathology
Presented March 6, 1990
Boston, Mass.

Presentor: Warren Maltzman

A NON ISOTOPIC HYBRID CAPTURE ASSAY FOR HIV NUCLEIC ACID SEQUENCES. L.S. Lee, H. Payne, C.-Y. Ou, G. Schochetman, and W. Maltzman, Enzo Biochem, New York, NY. and Centers for Disease Control, Atlanta, GA.

Oligonucleotide sequences were chosen from two relatively conserved regions of the human immunodeficiency virus type 1 (HIV-1) genome. These were then employed in a microciter plate assay which involves the capture of target nucleic acid by virtue of its complementarity to an immobilized capture oligonucleotide. Hybridization is detected by incubation with a second specific signal oligonucleotide, using an enzyme-dependent amplification system to yield a colorimetric readout. This strategy has been applied to detection of HIV DNA and RNA sequences in both model systems and clinical specimens.

In experiments where enzymatically amplified HIV DNA, from either the gag or env regions, was the target, we were able to detect HIV DNA in all (36/36) samples from seropositive individuals. These results were in agreement with a radioactive "gel assay" performed in parallel on the same samples. The results of reconstruction experiments in which known amounts of HIV DNA were assayed in our nonradioactive system, suggest that application of this technology to the problem of detection of HIV in clinical samples might allow the identification of individuals who harbor low levels of HIV proviral or viral nucleic acid, e.g prior to seroconversion. To date, we have detected HIV DNA after amplification in 6/6 samples taken from individuals who were seronegative at the time of sampling, but who subsequently seroconverted.

ABSTRACT FORM

Abstracts must be postmarked
by September 27, 1989

For additional abstract forms
call (404) 733-7550

PRESENTATION CHOICE

Poster only _____
Platform only _____
Either Poster or Platform ☒ _____

Stowell-Orbison _____
(application form enclosed).

Scientific Exhibit _____

CATEGORY (see listing on reverse)

Techniques

PRESS INTEREST

____ Yes ☒ No

ABSTRACT CHECKLIST

(Please Complete)

- ☒ Format corresponds to sample
- ☒ Completed Postcards enclosed
- ☒ Original and 3 photocopies enclosed
- ☒ Presentation choice checked
- ☒ Abstract fits within blue lines
- ☒ Abstract is typed directly on original form
- ☒ Abstract is not submitted for another meeting

MAIL ORIGINALS AND THREE (3)
PHOTOCOPIES TO:

United States and Canadian
Academy of Pathology
3643 Walton Way Extension
Augusta, GA 30909 U.S.A.

A NON ISOTOPIC HYBRID CAPTURE ASSAY FOR HIV NUCLEIC ACID SEQUENCES. L.S. Lee, H. Payne, C.-Y. Ou, G. Schochetman, and W. Maltzman, Enzo Biochem, New York, NY. and Centers for Disease Control, Atlanta, GA.

Oligonucleotide sequences were chosen from two relatively conserved regions of the human immunodeficiency virus type 1 (HIV-1) genome. These were then employed in a microtiter plate assay which involves the capture of target nucleic acid by virtue of its complementarity to an immobilized capture oligonucleotide. Hybridization is detected by incubation with a second specific signal oligonucleotide, using an enzyme-dependent amplification system to yield a colorimetric readout. This strategy has been applied to detection of HIV DNA and RNA sequences in both model systems and clinical specimens.

In experiments where enzymatically amplified HIV DNA, from either the gag or env regions, was the target, we were able to detect HIV DNA in all (36/36) samples from seropositive individuals. These results were in agreement with a radioactive "gel assay" performed in parallel on the same samples. The results of reconstruction experiments in which known amounts of HIV DNA were assayed in our nonradioactive system, suggest that application of this technology to the problem of detection of HIV in clinical samples might allow the identification of individuals who harbor low levels of HIV proviral or viral nucleic acid, e.g. prior to seroconversion. To date, we have detected HIV DNA after amplification in 6/6 samples taken from individuals who were seronegative at the time of sampling, but who subsequently seroconverted.

SIGNATURE: _____

SAMPLE FORMAT:

SAMPLE FORMAT FOR TYPING ABSTRACTS. J. Doe, Q. Smith, and Z. Jones, City University Medical College, Anywhere, AL and People's Hospital, Somewhere, CA

This is merely an example of the format to be used when typing an abstract which has multiple authors. Include only name of institution and city and state.

It is not necessary to skip a space between paragraphs. Just indent each paragraph three spaces.

Name and Address of Author for Correspondence:

W. Maltzman
Enzo Biochem
325 Hudson Street
New York, NY 10013

Office telephone number: (212) 741-3838

Name and address of non-members for
mailing meeting registration information:

REGISTRATION - Authors presenting the accepted scientific entries will be required to pay the general registration fee for the meeting. Meeting registration information is NOT automatically mailed to non-members. Please indicate above if you wish to receive this information.

hiroshima and Nagasaki ...
ed to gamma and neutron
Cancer (SCLC) made up 48% of
of the HN cases whereas
le up 34% of the HN cases and
Squamous cell carcinoma was
and 28% of the UMC. Among the
smoking in the HN but not the
tion of AC was lower in the HN

EPITOPE, D-14, IN BARRETT'S ESO-
AL ADENOCARCINOMA. JA Lapa MD* and
Naval Medical Center and *National
, Maryland.
as to evaluate the immunoperoxidase
hegale adenocarcinoma (EA), dysplastic
) and non-dysplastic Barrett's epithe-
out inflammatory atypia. In particu-
monoclonal antibody D-14, which is
specific CEA epitope expressed in colonic
adenocarcinoma. Four cases of EA, 4
NOBE (intestinal-type) and 5 cases of
stained by the avidin-biotin complex
era to D-14 (E-Z-EM), CEA (Dako),
keratins (Dako) and monoclonal low mo-
(Becton-Dickinson, Boehringer-Mannheim
Intensity and pattern of staining
necarcinomas showed 1-3+ luminal and
r D-14. Luminal staining (1-3+) was
DBE and 6 of 9 cases of NOBE. Only
plasmic staining for D-14 were seen in
ases, both NOBE. The other antibodies
failed to demonstrate differentiating
cs. We conclude that EA shows strong
with D-14, while DBE and NOBE do not.
eria for EA are not met, but strong D-14
is present, rebiopsy is recommended. The
d not help to differentiate DBE from NOBE.

BREAST CARCINOMAS - A COMPARISON BETWEEN
IMAGE ANALYSIS AND FLOW CYTOMETRY
W.M. Hamilton, B. Kamat, G.J. Heatley, L. Cook,
Medical Center, Burlington, MA., New
Hospital & Harvard Medical School, Boston, MA
terized image analysis (IA) and flow cytom-
ne DNA ploidy of 30 invasive breast carcin-
n-stained slides of touch preparations and
cytospin preparations were analyzed with
alyzer (CAS, Elmhurst, IL). FCM, using the
lter, Hialeah, FL), was performed on disag-
stained with propidium iodide. The results
FCM as the standard. The DNA indices meas-
chniques showed close correlation by linear
:(R=0.964, p<0.001). There were 16(53%)
aneuploid tumors, the latter consisting of
d and 2(14%) tetraploid tumors, and 3(21%)
aneuploid peaks. There was agreement be-
n 28 of 30(93%) tumors. A trend was observed
and negative estrogen receptor expression,
ade and mitotic rate, and lymphatic-vascular
ired smaller tissue samples, and permitted
visualization and selection of tumor cells.
ferred better resolution and greater sensi-
ng the presence of multiple aneuploid peaks,
mation on the S-phase. Overall, the two
ed comparable results and were complementary
DNA ploidy of breast carcinomas.

Results associated with chorionadmission of hyaline
abnormalities. Despite the negative correlation of hyaline
funisitis. Despite the negative correlation of hyaline
membrane disease and fetal pneumonia, there were 10 (10%)
patients with both conditions and 19 (20%) with hyaline
membrane disease and inflammation of membranes, cord, and/or
lung. In some instances, hyaline membrane disease was
attributable to oxygen therapy but in other cases, fetal
pneumonia and hyaline membrane disease coexisted, suggesting
a possible pathogenetic relationship.

327 EXPRESSION OF BLOOD GROUP ANTIGEN A (BGAA) EPITOPE ON
TUMOR CELLS: A FAVORABLE PROGNOSTIC FACTOR FOR SURGICALLY
RESECTED NON-SMALL CELL LUNG CANCER (NSCLC). J. Lee, J. Ro, A.
Sahin, W. Hittelman, B. Brown, C. Mountain, and W. Hong. M. D.
Anderson Cancer Center, Houston, TX

Previously, we reported that expression of epidermal growth
factor receptor (EGFR) on tumor cells, assessed by an anti-EGFR
monoclonal antibody 29.1 and the ABC immunoperoxidase technique,
is an important prognostic factor for patients (pts) with NSCLC
(Proc ASCO 8:226, 1989). However, this antibody was found to
cross-react with the BGAA epitope which prompted us to examine
the ABH blood group antigen expression on paraffin-embedded
NSCLC tumor sections using monoclonal antibodies for blood group
antigen A and B, and Ulex europaeus agglutinin I for H antigen.
Of 164 pts, who survived at least one month after surgery, 61
pts had a blood type A, 20 type B, 73 type O, and 10 type AB;
post-surgical stages were I in 68, II in 32, and III in 64 pts.
Of 71 pts with blood type A or AB, 42 (59%) pts who had BGAA
positive tumors survived significantly longer than the other 29
pts with BGAA negative tumors (p < .001) with a median survival
of 70 and 15 months, respectively. This difference was
independent of tumor stage, histologic grade, or cell types. In
comparison, a median survival for 93 pts with blood type B or
O was 39 months (p = .047). Expression of blood group antigen
B or H on tumor cells, however, was not a significant prognostic
factor. These data indicate that expression of BGAA epitope on
tumor cells is an important prognostic factor for NSCLC and it
might play an important role for the regulation of tumor growth.

328 A NON ISOTOPIC HYBRID CAPTURE ASSAY FOR HIV NUCLEIC
ACID SEQUENCES. L.S. Lee, H. Payne, C.-Y. Ou, G.
Schochetman, and W. Maltzman, Enzo Biochem, New York, NY. and
Centers for Disease Control, Atlanta, GA.

Oligonucleotide sequences were chosen from two relatively
conserved regions of the human immunodeficiency virus type 1
(HIV-1) genome. These were then employed in a microtiter plate
assay which involves the capture of target nucleic acid by
virtue of its complementarity to an immobilized capture
oligonucleotide. Hybridization is detected by incubation with
a second specific signal oligonucleotide, using an enzyme-
dependent amplification system to yield a colorimetric
readout. This strategy has been applied to detection of HIV
DNA and RNA sequences in both model systems and clinical
specimens.

In experiments where enzymatically amplified HIV DNA, from
either the gag or env regions, was the target, we were able to
detect HIV DNA in all (36/36) samples from seropositive in-
dividuals. These results were in agreement with a radioactive
"gel assay" performed in parallel on the same samples. The
results of reconstruction experiments in which known amounts of
HIV DNA were assayed in our nonradioactive system, suggest that
application of this technology to the problem of detection of
HIV in clinical samples might allow the identification of
individuals who harbor low levels of HIV proviral or viral
nucleic acid, e.g. prior to seroconversion. To date, we have
detected HIV DNA after amplification in 6/6 samples taken from
individuals who were seronegative at the time of sampling, but
who subsequently seroconverted.

a pericentral distribution in
immunohistochemical demonstration
of zone 3 hepatocytes in 5/8 trans
mates were negative. No dysplastic
found. At the level of expression
these transgenic mice, the HBV X
acting as an oncogene and its
requires further study.

330 * PRIMARY MALIGNANT LYMPHOC
IMMUNOHISTOCHEMICAL ANAL
W.J. Lenington, J. Greer, H. Schwartz, R.
University Medical Center, Nashville, TN.

Primary malignant lymphomas of bo
presenting in bone with no evidence o
reviewed 13 cases of primary ML of bon
open biopsy in 9 patients and Craig need
to 83 years of age with a male femu
examination, 9 cases were categorized
ML, and 3 cases were small cell ML. Dia
microscopy in 3 cases and by positive i
in all 13 cases.

Paraffin sections in all cases
immunoperoxidase panel: L-26; LN1;
Leu 22 (pan-T cell markers); and immu
and CD30 were added to the panel in
cell ML. 1 small non-cleaved ML, and
cell phenotypes (L-26, LN1, and/or LN
were identified in 3 of the 8-cell ML.
features (1 case: UCHL1+, Leu 22-; 1 ca
One T-cell ML was positive for CD15 ar
In summary, most primary ML of
and are at least intermediate grade,
by immunohistochemistry; however, i
T-cell phenotypes and plastic embed
wider variety of lymphoid antigens
generally satisfactory in open biopsi
Craig needle biopsies. Decalcificati
with paraffin immunoperoxidase de

331 LARYNGEAL AMYLOIDOSIS: A
REVIEW. J. Lewis, P. Kurt
Rochester, MN.

We have reviewed the clinicopa
of 22 cases of laryngeal amyloid
There were 11 males and 11 fem
years. Hoarseness was the most c
involvement were the false cord
concomitant tracheobronchial an
involvement was detected in one.

Grossly, these lesions pre
subglottal masses. Microscopic
amorphous, eosinophilic material
birefringence with the Congo re
performed in 19 cases. Eight
Lambda light chain was detected
show definite staining for l
beta-2-microglobulin were negati
plasma cells were always polycl
Ten patients underwent req
disease. One patient died
amyloidosis. In one case, a
systemic amyloidosis or hemato
Laryngeal amyloidosis is a
immunohistochemical studies sh
patients do not develop plas
usual clinical course is rel
persistent or recurrent respi

Abstract

Presented at US-Canadian Academy of Pathology Meeting
Boston, Massachusetts, March 1990

A NON-ISOTOPIC HYBRID CAPTURE ASSAY FOR HIV NUCLEIC ACID SEQUENCES.

L.S. Lee, H. Payne, C.-Y. Ou, G. Schochetman and W. Maltzman, Enzo Biochem, New York, NY and Centers for Disease Control, Atlanta, GA.

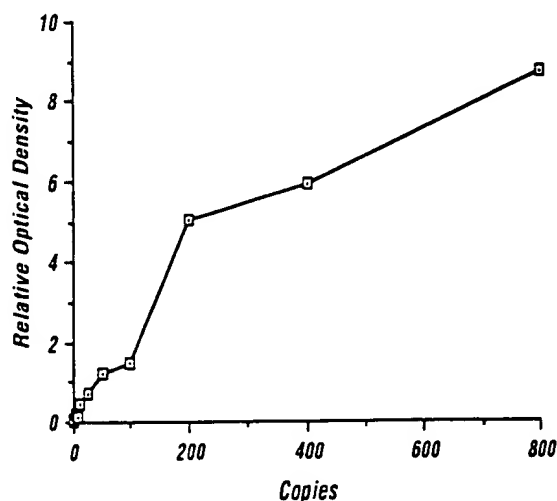
Oligonucleotide sequences were chosen from two relatively conserved regions of the human immunodeficiency virus type 1 (HIV-1) genome. These were then employed in a microtiter plate assay which involves the capture of target nucleic acid by virtue of its complementarity to an immobilized capture oligonucleotide. Hybridization is detected by incubation with a second specific signal oligonucleotide, using an enzyme-dependent amplification system to yield a colorimetric readout. This strategy has been applied to detection of HIV DNA and RNA sequences in both model systems and clinical specimens.

In experiments where enzymatically amplified HIV DNA, from either the gag or env regions, was the target, we were able to detect HIV DNA in all (36/36) samples from seropositive individuals. These results were in agreement with a radioactive "gel assay" performed in parallel on the same samples. The results of reconstruction experiments in which known amounts of HIV DNA were assayed in our nonradioactive system suggest that application of this technology to the problem of detection of HIV in clinical samples might allow the identification of individuals who harbor low levels of HIV proviral or viral nucleic acid, *e.g.*, prior to seroconversion. To date, we have detected HIV DNA after amplification in 6/6 samples taken from individuals who were seronegative at the time of sampling, but who subsequently seroconverted.

Figure 2.

DETECTION OF HIV SEQUENCES IN AMPLIFIED SAMPLES OF CONTROL REACTIONS

Sample	Copies	Relative Optical Density
2393	800,000	93.45
2410	80,000	38.28
2398	8,000	15.38
2391	800	8.74
2390	400	5.91
2395	200	5.03
2400	100	1.44
2394	50	1.19
2403	25	0.682
2397	12.5	0.455
2399	6.25	0.104
2402	3.12	0.170
2392	1.56	0.038
2396	0.00	0.014
water	0.00	0.003



Samples represented 1 µg of human DNA which was amplified for 35 rounds in the presence of the indicated number of copies of cloned HIV DNA. Relative optical density was read at the termination of the detection reaction and normalized to 2 µl of the undiluted amplification reaction product. In all cases OD's of greater than 1.00 were based upon assays of diluted samples that gave OD readings between 0.1 and 1.0.